

## WHAT IS CLAIMED IS:

1. A method for the bioproduction of C<sub>6</sub> to C<sub>22</sub> mono- and di-carboxylic acids comprising
- contacting, under aerobic conditions, a transformed *Pichia pastoris* characterized by a genetically-engineered alkane hydroxylating activity with at least one C<sub>6</sub> to C<sub>22</sub> straight chain hydrocarbon; and
  - recovering the C<sub>6</sub> to C<sub>22</sub> mono- and di-carboxylic acids.
2. The method of Claim 1 wherein the transformed *Pichia pastoris* is strain SW 64/65 identified as ATCC 74409; the at least one C<sub>6</sub> to C<sub>22</sub> straight chain hydrocarbon is dodecane; and the product recovered is dodecanedioic acid.
3. A transformed *Pichia pastoris* comprising
- at least one copy of a foreign gene encoding cytochrome P450 monooxygenase; and, optionally,
  - at least one copy of a foreign gene encoding cytochrome P450 reductase,
- each gene operably linked to suitable regulatory elements such that alkane hydroxylating activity is enhanced upon contact with at least one C<sub>6</sub> to C<sub>22</sub> straight chain hydrocarbon.
4. The transformed *Pichia pastoris* of Claim 3 wherein the foreign gene encoding cytochrome P450 monooxygenase is selected from the group consisting of Alk1-A (D12475), Alk2-A (X55881), Alk3-A (X55881), Alk4-A (D12716), Alk5-A (D12717), Alk6-A (D12718), Alk7 (D12719), and Alk8 (D12719).
5. The transformed *Pichia pastoris* of Claim 3 wherein the foreign gene encoding cytochrome P450 reductase is cytochrome P450 reductase (D25327).
6. A transformed *Pichia pastoris* strain characterized by an enhanced alkane hydroxylating activity and comprising,
- at least one DNA fragment from *Candida maltosa* ATCC 90677 selected from the group of DNA fragments encoding cytochrome P450 monooxygenase Alk1-A and cytochrome P450 monooxygenase Alk3-A; and, optionally,
  - at least one DNA fragment from *Candida maltosa* ATCC 90677 encoding cytochrome P450 reductase,
- each DNA fragment operably linked to suitable regulatory elements such that alkane hydroxylating activity is enhanced upon contact with at least one C<sub>6</sub> to C<sub>22</sub> straight chain hydrocarbon.
7. A transformed *Pichia pastoris* strain SW64/65 identified as ATCC 74409.
8. A method for the enhanced bioproduction of C<sub>6</sub> to C<sub>22</sub> mono- and di-carboxylic acids comprising

a) contacting, under aerobic conditions, a transformed *Candida maltosa* characterized by a genetically-engineered, enhanced alkane hydroxylating activity with at least one C<sub>6</sub> to C<sub>22</sub> straight chain hydrocarbon; and

b) recovering the C<sub>6</sub> to C<sub>22</sub> mono- and di-carboxylic acids.

5           9.   The method of Claim 8 wherein

a) the genetically-engineered, enhanced alkane hydroxylating activity arises from

i) at least one additional copy of the genes encoding cytochrome P450 monooxygenase selected from the group consisting of Alk1-A (D12475), Alk2-A (X55881), Alk3-A (X55881), Alk4-A (D12716), Alk5-A (D12717), Alk6-A (D12718), Alk7 (D12719), and Alk8 (D12719); or

ii) at least one additional copy of the gene encoding cytochrome P450 reductase (D25327); or

iii) at least one additional copy of both the genes of i) and ii);

15           b) the at least one C<sub>6</sub> to C<sub>22</sub> straight chain hydrocarbon is dodecane;

and

c) the product recovered is dodecanedioic acid.

10.   A transformed *Candida maltosa* comprising

a) at least one additional copy of a gene encoding cytochrome P450 monooxygenase; or

b) at least one additional copy of a gene encoding cytochrome P450 reductase; or

c) at least one additional copy of both the gene encoding P450 monooxygenase and the gene encoding cytochrome P450 reductase,

each gene operably linked to suitable regulatory elements such that alkane hydroxylating activity is enhanced upon contact with at least one C<sub>6</sub> to C<sub>22</sub> straight chain hydrocarbon.

11.   The transformed *Candida maltosa* of Claim 10 wherein the genes encoding cytochrome P450 monooxygenase are selected from the group consisting of Alk1-A (D12475), Alk2-A (X55881), Alk3-A (X55881), Alk4-A (D12716), Alk5-A (D12717), Alk6-A (D12718), Alk7 (D12719), and Alk8 (D12719).

12.   The transformed *Candida maltosa* of Claim 10 wherein the gene encoding cytochrome P450 reductase is cytochrome P450 reductase (D25327).

13.   A transformed *Candida maltosa* strain comprising

a) at least one DNA fragment from *Candida maltosa* (ATCC 90677) selected from the group of DNA fragments encoding cytochrome P450 monooxygenase ALK1-A and cytochrome P450 monooxygenase ALK3-A, and

b) at least one DNA fragment from *Candida maltosa* (ATCC 90677) encoding cytochrome P450 reductase, each gene operably linked to suitable regulatory elements such that alkane hydroxylating activity is enhanced upon contact with at least one C<sub>6</sub> to C<sub>22</sub> straight chain hydrocarbon.

14. A method for the enhanced bioproduction of C<sub>6</sub> to C<sub>22</sub> mono- and di-carboxylic acids comprising

a) contacting, under aerobic conditions, transformed *Candida maltosa* characterized by a genetically-engineered, blocked  $\beta$ -oxidation pathway with at least one C<sub>6</sub> to C<sub>22</sub> straight chain hydrocarbon; and

b) recovering the C<sub>6</sub> to C<sub>22</sub> mono- and di-carboxylic acids.

15. The method of Claim 14 wherein the transformed *Candida maltosa*  $\beta$ -oxidation pathway is functionally blocked by disruption of both POX4 genes encoding acyl-CoA oxidase.

16. A transformed *Candida maltosa* characterized by a  $\beta$ -oxidation pathway functionally blocked by disruption of both POX4 genes encoding acyl-CoA oxidase.

17. A transformed *Candida maltosa* characterized by a  $\beta$ -oxidation pathway functionally blocked by disruption of both POX4 genes encoding acyl-CoA oxidase using a single URA3 selectable marker.

18. A transformed *Candida maltosa* strain SW81/82 identified as ATCC 74431.

19. A method for the enhanced bioproduction of C<sub>6</sub> to C<sub>22</sub> mono- and di-carboxylic acids comprising

a) contacting, under aerobic conditions, transformed *Candida maltosa* characterized by

i) a genetically-engineered, enhanced alkane hydroxylating activity, and

ii) a genetically-engineered, blocked  $\beta$ -oxidation pathway, with at least one C<sub>6</sub> to C<sub>22</sub> straight chain hydrocarbon; and

b) recovering the C<sub>6</sub> to C<sub>22</sub> mono- and di-carboxylic acids.

20. A transformed *Candida maltosa* characterized by

a) an enhanced alkane hydroxylating activity arising from

i) at least one additional copy of a gene encoding cytochrome P450 monooxygenase selected from the group consisting of Alk1-A (D12475), Alk2-A (X55881), Alk3-A (X55881), Alk4-A (D12716), Alk5-A (D12717), Alk6-A (D12718), Alk7 (D12719), and Alk8 (D12719), or

ii) at least one additional copy of a gene encoding cytochrome P450 reductase (D25327), or

Sub  
A5

iii) at least one additional copy of both the genes i) and ii); and  
b) a  $\beta$ -oxidation pathway functionally blocked by disruption of both  
POX4 genes encoding acyl-CoA oxidase.

Sub  
A6

21. The transformed *Candida maltosa* strain of Claim 20 wherein the  
enhanced alkane hydroxylating activity of a) arises from DNA fragments  
encoding cytochrome P450 monooxygenase ALK1-A and cytochrome P450  
monooxygenase ALK3-A.

22. A transformed *Candida maltosa* strain SW84/87.2 identified as  
ATCC 74430.

23. The method of Claims 1, 8, 14, or 19 wherein the at least one C<sub>6</sub> to  
C<sub>22</sub> straight chain hydrocarbon is selected from the group consisting of hexane,  
heptane, octane, nonane, decane, undecane, dodecane, tridecane, tetradecane,  
pentadecane, hexadecane, heptadecane, octadecane, nonadecane, eicosane,  
reneicosane, docosane and their respective mono-carboxylic acids and esters.

24. A DNA fragment comprising a) a first *Candida maltosa* promoter  
operably linked to DNA encoding at least one polypeptide from *Candida maltosa*  
and b) a second *Candida maltosa* promoter operably linked to DNA encoding at  
least one polypeptide from *Candida maltosa*.

25. A DNA fragment comprising a) a first *Candida maltosa* promoter  
operably linked to a gene encoding a *Candida maltosa* cytochrome P450  
monooxygenase and b) a second *Candida maltosa* promoter operably linked to a  
gene encoding a *Candida maltosa* cytochrome P450 reductase.

26. A DNA fragment comprising a) a first *Candida maltosa* PGK  
promoter which is operably linked to a gene encoding cytochrome P450  
monooxygenase selected from the group consisting of Alk1-A (D12475), Alk2-A  
(X55881), Alk3-A (X55881), Alk4-A (D12716), Alk5-A (D12717), Alk6-A  
(D12718), Alk7 (D12719), and Alk8 (D12719) and b) a second *Candida maltosa*  
PGK promoter operably linked to a gene encoding a *Candida maltosa* cytochrome  
P450 reductase.

27. A plasmid selected from the group consisting of pSW84 and pSW87.

Sub  
A7

09115502.071698